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SODIUM TELLURITE AS A RAPID TEST FOR THE VIABILITY OF TUBERCLE BACILLI

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS, XIII*

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A reliable agent which would indicate in a short period of time life or death of the tubercle bacillus and eliminate the animal test would be a valuable help in work on tuberculosis, especially on its experimental chemotherapy. It was hoped that such an agent was at hand when Gosio described his selenite and tellurite reduction tests as indicators of life or death of bacteria. In his tests, he included the tubercle bacillus and found that it behaved exactly as other bacteria towards the agent. If Gosio's statement is correct, one could accomplish within twenty-four to forty-eight hours what would require at least a month by the inoculation method. Since Gosio's tests on the tubercle bacillus, however, were only incidental in a large series, it was necessary in determining their practical value to control his results and to apply them to actual experiments in chemotherapy. If the tests could not be used in chemotherapeutic experiments where interfering chemical substances are dealt with, they perhaps could be an aid in the determination of whether a culture to be used in an experiment was viable or not. Absence of this information frequently occasions the loss of valuable time because where the culture is not viable, this becomes apparent only at the end of a month or so when neither controls nor test animals develop tuberculosis, or when, in culture experiments, no growth occurs. Further investigation was also indicated by the fact that the agents in question possess possible chemotherapeutic value.

LITERATURE

As a result of his work with the alkali selenites and tellurites, Gosio[1] concludes that these salts are valuable reagents for determining bacterial life as they are reduced by living bacteria, the reduction products staining the bacteria cells. The tellurites show a black stain, the selenites a red. He preferred

* Received for publication October 10, 1914.

1. Ztschr. f. Hyg. u. Infektionskh., 1905, 51, p. 65.

the tellurites as they are more permanent and because they are not so easily mistaken for interfering colors. To obtain the best action, it is essential that the microorganisms are growing well; when spores are forming or development is at a standstill, the test is uncertain and unreliable. The delicacy of the reaction is in direct relation to the amount of chemical reagent and the quantity of bacteria that can live in its presence. Therefore, it is essential that the chemical should not reach a dose toxic to the bacteria. All factors that increase the activity of the bacteria increase the reaction with the indicator, and vice versa. Not all media are equally favorable for the reaction; broth and milk proved best, serum inhibited the reaction, while a small amount of sugar increased it. Dead bacilli do not appreciably decompose the tellurite, for instance, with typhoid bacilli. With some bacteria, long contact and higher temperature often produce an ash-gray color. The salt acts plainly in dilutions of 1:100,000 and even 1:200,000, and retains its chemical properties for months in ordinary substrata in which it may be used. Aseptic reduction can occur, but only under extraordinary conditions (in the presence of reducing agents, high heat, vacuum, etc.). Injected into tissues of living animals, methyl telluride with its characteristic odor is formed, and all the nuclei and part of the cytoplasm are stained black with tellurium. Gosio examined one hundred and seventy-three microorganisms and divided them into three classes dependent upon the reaction obtained; (1) a decided reaction, (2) a less intense, but fully evident, reaction [in this class he placed a bovine, a human, and an avian tubercle bacillus and a so-called psuedotuberculosis bacillus (Rabinowitsch)], (3) very slight reaction.

Belfanti[2] studied the behavior of the reaction of Gosio with tubercle bacilli by adding to agar plates small amounts of tellurite (1:25,000-1:50,000) and found that lumps of culture of human, bovine and avian bacilli reduced the potassium tellurite markedly and, in a few hours, the bacilli were stained black. The reaction occurred within wide ranges of temperature; it was most marked at 37 C., was slightly less at room temperature, and developed slowly on ice. High concentrations (1 percent and 0.1 percent) of the tellurium salt destroyed the organisms, so that when transferred to suitable nutrient media they did not grow, or produced only a slight growth. Cultures exposed for a few minutes to ether or acetone vapors did not give the reaction. Belfanti suggested that these salts are bacteriotropic in the Ehrlich sense, and can be used for therapeutic study.

If the tellurite test for viability of tubercle bacilli is to be used in chemotherapeutic work, it must indicate the life or death of the bacilli after they have been treated with the chemical which is being tested for its tuberculocidal action. Under ideal conditions, it should be possible to add the tellurite directly to the chemical solution and obtain the desired test. Practically, however, this is impossible, as the tellurite is rather active chemically and is susceptible to decomposition or precipitation by a large number of substances. If the test could be carried out satisfactorily in distilled water or in 0.9 percent salt solu-

2. Rev. Ist. Lomb. di sc. e lett rendic, Milano, 1912, 45, p. 539; Ztschr. f. Chemoth., Orig., 1912, p. 113.

tion, this objection would not be as great, but, as was pointed out by Gosio and will be shown in this paper, the reduction test is dependent, not so much upon life or death of the organism, as it is upon the fact that the organism is in active metabolism and present in sufficient number. Therefore, to obtain the test the organism must be suspended in a suitable nutrient medium. Another course is open and that is to suspend the tubercle bacilli in the solution to be tested for its tuberculocidal action, wash the bacilli free from this solution with sterile salt solution or distilled water, add the tellurite in sterile broth to the washed bacilli, and then incubate. This would prove ideal if it were not for the fact that the washing process and the centrifugation can rarely be carried out without contamination by rapidly growing organisms which also reduce the tellurite and have the ability of rapidly overgrowing the tubercle bacilli. As will be shown later, tubercle bacilli which have been emulsified are very slow in reducing the tellurite or may even not do so unless they are present in very large amounts.

In spite of the fact that the tellurite test could not be used as a reliable index of life or death of the tubercle bacillus in tuberculocidal experiments, it nevertheless proved of value as a rapid index of life or death of cultures of tubercle bacilli if the tests were properly carried out. The following experiments led to a method which would reveal in from one to two hours whether a culture of tubercle bacillus was viable or not.

A series of tubes were made containing 5 c.c. of 0.9 percent salt solution and dilutions of sodium tellurite 1:1,000, 1:1,500, 1:7,500, 1:10,000, 1:15,000, 1:75,000, 1:100,000, 1:150,000, and 1:750,000. To each of these were added a few drops of a fairly heavy emulsion of tubercle bacilli in salt solution, containing 5-10 mg. bacilli to 10 c.c. of the solution, and placed in the incubator at 37 C. No visible reduction occurred in any of these within seventy-two hours. Similar dilutions were then made and to them were added, instead of an emulsion, lumps of tubercle bacillus cultures. They were placed at 37 C. No visible reduction occurred in any of the dilutions up to 1:100,000 in seventy-two hours; 1:150,000 gave a slight reduction in forty-eight hours, and this only in a lump of culture on the surface of the liquid, and 1:750,000 gave a good reduction, but not until after forty-eight hours.

Emulsified tubercle bacilli in glycerin broth, unless present in larger amounts with the same dilutions of tellurite used above, gave about similar results as with the salt solution. Lumps of culture in glycerin broth gave a reduction in dilutions from 1:100,000, etc., but not appearing until after about twenty-four hours at 37 C.

If the tubercle bacilli are present in sufficient amount, a reduction of the tellurite is obtained even in salt solution. To 3 c.c. of a heavy emulsion of

tubercle bacilli were added varying amounts, 0.01, 0.05, 0.1, and 0.2 mg. of sodium tellurite. This was incubated at 37 C. A reduction occurred after twenty-four hours in all except the one containing 0.01 mg.

A series of sterile, hollow, ground glass slides were prepared and in the bowl of each a small lump of culture of tubercle bacillus was placed. To each was added a drop of varying concentrations (1 : 500, 1 : 5,000, 1 : 50,000, etc.) of sterile sodium tellurite in distilled water and covered by means of a sterile cover glass bordered with sterile vaselin to prevent drying of the culture. The slides were then placed in the incubator at 37 C. The one to which had been added one drop of 1 : 500 sodium tellurite gave a reduction within thirty minutes to one hour, being completely black in a few hours. The 1 : 5,000 sodium tellurite test gave only a faint reduction as compared to the 1 : 500, not appearing before twelve to twenty-four hours, and the 1 : 50,000 did not give a visible reduction even in forty-eight to seventy-two hours.

Two questions immediately arose as a result of these experiments: Whether or not emulsified bacteria could, by some means, be concentrated and the concentrated residue be used for this rapid test, for tuberculocidal work (contaminators would not develop rapidly enough to interfere with a test occurring in so short a time) : and whether or not this test was really an index of viability of the organism.

The former question was answered as follows: A heavy emulsion of tubercle bacilli was made and concentrated by placing, drop by drop, on a small, sterile, unglazed porcelain plate until a fairly large accumulation of bacilli had been obtained. To these were added a few drops of 1 : 500, 1 : 5,000, and 1 : 50,000 sodium tellurite in distilled water, but no reduction was observed within forty-eight hours at 37 C.

Whether or not this test was really an index of viability of the tubercle bacillus was determined as follows: Two well-grown cultures of human tubercle bacilli on glycerol agar were exposed to the light of a 32 c. p. tungsten electric light in the incubator at 37 C., and several small lumps of these cultures were tested before and every twelve hours after exposure to the light by the tellurite drop test, and at the same time by inoculation into normal guinea-pigs. On the third day and later after exposure to the electric light, the lumps of culture gave a negative drop tellurite test, and, coincident with this, failed to produce tuberculosis in the guinea-pigs, whereas a lump taken from a control tube kept under the same conditions, but wrapped in heavy black paper to keep out the light, still reduced the tellurite and produced tuberculosis in guinea-pigs within seven days.

Belfanti suggested that the selenites and tellurites may be of value as a basis for a chemotherapy for tuberculosis. He did not, however, report any observations as a support for his statement. With a view to breaking ground in this direction, sodium tellurite was tested, so far as this was possible, for its value as a chemotherapeutic agent in tuberculosis. A few objections may immediately be made to its use; its ready reduction by the more active cells of the animal organism in comparison to its slow reduction by the rather inactive tubercle bacillus, and its chemical instability. Even these apparent disadvantages may become advantages under certain conditions, if, for instance, the

unreduced tellurite revealed a highly tuberculocidal action while practically non-lethal to the animal organism because of its ready power to reduce it. On account of the changes produced in the compound by introduction into the animal organism, the test for chemotherapeutic value naturally becomes more complicated. The following experiments are not conclusive, but can be used merely as an aid in carrying out further work with the selenites and tellurites.

As a gauge of the toxicity of a compound, such as, sodium tellurite, which is decomposed and reduced by the living tissues with which it comes in contact,[3] it seems that the intravenous lethal dose is the only fair index for our purpose. For this reason, the toxicity of sodium tellurite was tested intravenously in rabbits with the results shown in Table 1.

TABLE 1

TOXICITY OF SODIUM TELLURITE INJECTED INTRAVENOUSLY IN RABBITS*

Rabbit	Weight of Animal in Grams	Amount Injected in Cubic-centimeters	Concentration of Sodium Tellurite Percentage	Amount Milligrams per Kilo	Result
1	1,350	5.0	0.01	0.37	Lived
1 Third day.	....	1.0	0.1	0.74	Lived
2	2,350	1.5	0.1	0.64	Lived
2 Fifth day..	....	2.0	0.1	0.85	Dead in 16 hours
3	2,350	2.0	0.1	0.85	Dead in 12 hours
4	1,450	1.5	0.1	1.03	Dead in 12 hours
5	1,150	2.0	0.1	1.74	Dead in 24 hours
6	1,130	2.0	0.1	1.77	Dead in 12 hours
7	1,250	1.0	1.0	8.00	Dead in 20 minutes

* In guinea-pigs (260-350 gm.), Gosio found that 10.0 mg. potassium tellurite, given subcutaneously in 5-10 c.c. distilled water or blood serum, were fatal in seven hours, 3 mg. in forty-eight hours, and 0.1-2.0 mg. produced only local necrosis and induration.

In experiments on mice, 0.2 mg. sodium tellurite intraperitoneally was found lethal to a 10 gm. mouse, whereas 0.02 and 0.04 mg. were not.

These experiments show that the intravenous lethal dose of sodium tellurite to rabbits is about 0.8 mg. per kilo body weight.

In order to determine whether sodium tellurite possessed any power to inhibit growth or had a tuberculocidal effect, a number of experiments were performed.

A series of glycerol agar tubes were made containing from 0.01-0.1 mg. sodium tellurite to 10 c.c. These were inoculated with human tubercle bacilli, placed in an incubator at 37 C., and observed at frequent intervals. After two months, all the tubes, controls, and tests revealed a good growth; the dilutions containing 0.05-0.1 mg. sodium tellurite revealed a definite reduction (blackening) of the original transplant and a dark gray color of the new growth, the edges being colorless in places, and the dilutions containing 0.01-0.05 mg. revealed a reduc-

3. Mead and Gies: Am. Jour. Physiol., 1901-2, 5, p. 104.

tion (slight in the higher dilutions) by the original transplant, but the new growth was not even gray.

A series of tubes were made, each containing 2 c.c. of a uniform emulsion of human tubercle bacilli in 0.9 percent salt solution and varying amounts of sodium tellurite (0.01, 0.05, 0.1, and 0.2 mg.). These were placed in the incubator at 37 C. for forty-eight hours and then were injected into normal guinea-pigs. All the guinea-pigs developed tuberculosis.

As a result of these experiments, it can be stated that sodium tellurite, in amounts up to 0.1 mg. in 10 c.c. glycerol agar, does not inhibit or prevent the growth of the human tubercle bacillus; in amounts up to 0.2 mg. in 2 c.c. salt solution for forty-eight hours at 37 C., it does not kill the tubercle bacillus.

TABLE 2

EXPERIMENTS ON TUBERCULOCIDAL ACTION AND REDUCTION OF SODIUM TELLURITE

Concentration of Sodium Tellurite	Appearance of Culture Before Incubation	Results of Incubation	Result of Inoculation
0.1, A	Clear	Slight gray sediment	Dead 24 hours after injection
0.1, B	Turbid (ppt)	Heavy gray sediment	Dead 48 hours after injection
0.05, A	Clear	Slight gray sediment	Dead 24 hours after injection
0.05, B	Turbid (ppt)	Heavy gray sediment	Dead 24 hours after injection
0.01, A	Clear	Definite, slight gray sediment	Marked, generalized tuberculosis in 85 days
0.01, B	Clear	Fairly distinct, gray sediment	Marked, generalized tuberculosis in 48 days
0.005, A	Clear	Sediment, very slight gray	Marked, generalized tuberculosis in 85 days
0.005, B	Clear	Fairly distinct, gray sediment	Fairly advanced tuberculosis in 33 days
0.001, A	Clear	Very faint gray, indistinct sediment	Fairly advanced tuberculosis in 49 days
0.001, B	Clear	Fairly distinct, gray sediment	Well-advanced, generalized tuberculosis in 67 days
0.0001, A	Clear	Faint, questionable sediment	Well-advanced, generalized tuberculosis in 85 days
0.0001, B	Clear	Fairly distinct, dark gray sediment	Well-advanced, generalized tuberculosis in 85 days
Control 1, A	Clear	Faint gray sediment	Mild, generalized tuberculosis in 85 days
Control 2, A	Clear	Faint gray sediment	Fairly marked, generalized tuberculosis in 85 days
Control 1, B	Clear	Faint gray sediment	Marked, generalized tuberculosis in 55 days
Control 2, B	Clear	Faint gray sediment	Mild, generalized tuberculosis in 85 days

Thus far the tellurite has been tested only for its tuberculocidal action in fairly high dilutions, and the bactericidal action of the tellurite has been studied independent of whether it was reduced or not. In the hope of correlating these a little more, the following experiments were carried out. A much heavier emulsion of tubercle bacilli was used than had been used in most of the previous experiments, and one that should give an unquestionable reduction, if possible.

Two series of tubes were made, Series A containing 5 c.c. of 0.9 percent salt solution, and Series B, 5 c.c. of 5 percent glycerol broth, in which were suspended heavy emulsions of human tubercle bacilli, 2 mg. in each tube. To these were added varying amounts of sodium tellurite, making concentrations of 0.0001, 0.001, 0.005, 0.01, 0.05, and 0.1 percent. Two controls were made with 5 c.c. of 0.9 percent salt solution and two with 5 c.c. of 5 percent glycerol broth, containing a similar amount of emulsified human tubercle bacilli but without sodium tellurite. The tubes were all placed in the incubator at 37 C. for forty-eight hours, and injected into normal guinea-pigs at the end of this time. The results are given in Table 2.

GENERAL SUMMARY

As a result of an attempt to use the Gosio vital reaction (sodium tellurite) as an index of life of virulent human tubercle bacilli in bactericidal experiments in connection with chemotherapeutic work, it may be stated that it was not found to be an available general reagent for this purpose, at least by the methods tested.

Nevertheless, by its use a simple, rapid test was developed for determining the viability of cultures of tubercle bacilli, of value especially in eliminating such loss of time as may be occasioned by working with dead instead of viable cultures. A small lump of the culture to be tested is placed in the cup of a sterile, hollow glass slide and one or two small drops of sterile 0.2 percent sodium tellurite in distilled water are added; it is covered with a sterile glass cover slip bordered with sterile vaselin, and placed in the incubator at 37 C. Life of the organism is indicated by the blackening of the lump of culture, which occurs in from thirty minutes to two hours.

Sodium tellurite is lethal to rabbits when it is given intravenously in amounts of about 0.8 mg. per kilo. It does not kill the tubercle bacillus even when in 0.01 percent concentration in salt solution or glycerol broth for forty-eight hours at 37 C., nor does it inhibit the growth in 0.001 percent concentration on glycerol agar.